

## Research paper

Physiological and biochemical changes induced by drought stress during the stem elongation and anthesis stages in the *Triticum* genusSumitra Pantha<sup>a,b</sup>, Benjamin Kilian<sup>c</sup>, Hakan Özkan<sup>d</sup>, Frederike Zeibig<sup>a</sup>, Michael Frei<sup>a,1,\*</sup><sup>a</sup> Department of Agronomy and Crop Physiology, Institute of Agronomy and Plant Breeding I, Justus Liebig University, Heinrich-Buff-Ring 26, Giessen 35392, Germany<sup>b</sup> National Plant Breeding and Genetics Research Centre, Nepal Agricultural Research Council, Khumaltar Lalitpur, Kathmandu P.O. Box 1135, Nepal<sup>c</sup> Global Crop Diversity Trust, Platz Der Vereinten Nationen 7, Bonn 53113, Germany<sup>d</sup> Hakan Özkan, Department of Field Crops, Faculty of Agriculture, Çukurova University, Adana 01330, Turkey

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## ABSTRACT

Drought stress negatively influences the growth, development, and grain yield of wheat by disrupting its morphological, physiological, and biochemical processes. This study examined the effects of drought stress during the stem elongation and anthesis developmental stages of species within the *Triticum* genus along with their drought adaptation mechanisms under fully watered and drought conditions. We tested the following two hypotheses: (1) drought tolerance mechanisms for osmotic and stomatal regulation that lead to oxidative stress are correlated between the stem elongation and anthesis stages and affect grain yield loss, and (2) compared with modern cultivars, wild wheat cultivars exhibit greater drought tolerance. To test these hypotheses, a greenhouse pot experiment was conducted using 17 genotypes of wild wheat relatives and landraces, with modern cultivars included for comparison. Drought stress was induced during the stem elongation and anthesis stages until the average soil moisture was approximately 15 % and 18 %, respectively, of the pot's water holding capacity. The soil moisture was maintained at 80–90 % for the fully watered treatment. An examination of physiological and biochemical traits revealed that drought significantly reduced stomatal conductance (gsw) and relative water content (RWC) during both developmental stages. However, significant increases occurred in the malondialdehyde (MDA) content during both stages and in the proline content during the anthesis stage. Drought stress significantly decreased the number of days to heading and anthesis, indicating that drought escape occurs under severe drought stress. Furthermore, drought significantly decreased morphological and yield-related traits, with the greatest reduction (51 %) occurring in grain yield. Weakly significant positive associations of biochemical and some physiological traits between the stem elongation and anthesis stages partially confirmed our first hypothesis, whereas our results relating to the second hypothesis were inconclusive. We observed genotype-dependent responses to drought stress during both stages for various measured traits. No associations of RWC, proline, or MDA with grain yield were found. However, stomatal conductance was negatively correlated with grain yield under drought stress at the anthesis stage. Certain wild wheat genotypes and landraces exhibited drought avoidance, escape, and tolerance mechanisms, which positively contributed to grain yield. We identified *T. monococcum* subsp. *sinskajae*, *T. boeoticum* and *T. dicoccoides* as the most drought-tolerant genotypes. The findings of this study provide important insight for understanding the drought adaptation traits and their use in wheat breeding programs.

**Abbreviations:** ARI2, anthocyanin reflectance index 2; BM, aboveground biomass; CRI2, carotenoid reflectance index 2; DTA, days to anthesis; DTH, days to heading; DTM, days to maturity; ET, effective tillers; GN, grain numbers; Gsw, stomatal conductance; GY, grain yield; HI, harvest index; Lic2, Lichtenthaler index 2; MCARI1, modified chlorophyll absorption reflectance index 1; MDA, malondialdehyde; NDVI, normalized difference vegetation index; PCA, principal component analysis; PEL, peduncle exertion length; PH, plant height; PhiPS2, photosystem II efficiency; PWHC, pot soil water-holding capacity; RWC, relative water content; SL, spike length; SHL, shoot length; TT, total tillers; TKW, thousand kernel weight.

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## 1. Introduction

The frequency and severity of drought stress have increased due to climate change, which poses a serious threat to crop production globally (Munné-Bosch and Villadangos, 2023; Shehzad et al., 2023; Vicente-Serrano et al., 2022). Drought stress induces morphological, physiological, biochemical, and molecular changes in plants (Sallam et al., 2019; Shelake et al., 2022). These changes affect plant growth and development, substantially reducing crop yields (Farooq et al., 2014; Shelake et al., 2022). The severity of drought stress depends on its intensity and frequency, as well as on the crop's phenological stage (Chaouachi et al., 2023; Wang et al., 2022). The anthesis and grain-filling developmental stages in wheat are most sensitive to drought stress, which affects grain yield (Sarto et al., 2017; Shokat et al., 2023). However, wheat plants can be affected by drought stress at any growth stage, including seedling, tillering, and stem elongation (Sallam et al., 2019; Wang et al., 2015). When plants are exposed repetitively to drought stress, they may develop drought stress memory, which enables them to respond faster to further water stress, thus providing greater protection (Jacques et al., 2021; Wang et al., 2014, 2015). However, some biochemical and physiological changes caused by repetitive and severe droughts could be ineffective in providing protection (Krasensky and Jonak, 2012). In addition, severe drought stress events at the stem elongation stage affect leaf initiation and expansion, reducing plant biomass and the number of grains per spike and ultimately limiting grain yield (Blum et al., 1990; Saeidi et al., 2015).

The negative impacts of drought stress caused by cell dehydration include restricted cell elongation, stomatal closure and a reduction in photosynthetic activity, thereby inhibiting plant growth and development (McAusland et al., 2020; Chaouachi et al., 2023; Tambussi et al., 2002). The initial response of plants to drought stress is stomatal closure, which is beneficial for their survival under drought, as it reduces water loss from leaves (Nolan et al., 2017). However, this causes a decrease in the CO<sub>2</sub> assimilation rate, so plants can absorb more light than they actually use for photosynthesis (Li et al., 2017). Consequently, drought stress triggers the production of reactive oxygen species resulting from the impairment of photochemical reactions in the chloroplast, which leads to oxidative stress in the plant and damage to cell membranes due to lipid peroxidation (Abid et al., 2018; Hayat et al., 2012).

The drought stress response in plants is a complex phenomenon that involves changes in plant morphology and physiology and in biochemical pathways (Bhatta et al., 2018; Shelake et al., 2022). This process depends on a range of adaptive mechanisms, which include escape from drought as well as drought avoidance and tolerance (Bapela et al., 2022; Farooq et al., 2014; Sallam et al., 2019). The escape strategy involves accelerated crop phenology or a shortening of the plants' growth period to complete their life cycle (Shavrukov et al., 2017). Drought avoidance is a water-saving strategy involving different morphophysiological changes, such as a reduction in stomatal conductance by lowering water use or preventing dehydration (Bandurska, 2022; Martínez-Vilalta and García-Fórner, 2017). Plants also exhibit an avoidance strategy characterized by reduced plant growth and development, which results in decreases in plant height, tiller number, and biomass (Anyia and Herzog, 2004; Izanloo et al., 2008; Zia et al., 2021).

A drought tolerance strategy involves the regulation of physiological and biochemical changes in plants (Farooq et al., 2014; Sallam et al., 2019). This process mainly hinges on osmotic adjustments to maintain cell volume and turgor pressure, enabling plants to continue growing under drought stress (Izanloo et al., 2008; Nolan et al., 2017). Osmotic adjustment lowers the osmotic potential and maintains the water status of plants, thereby maintaining their cellular functions during osmotic stress (Abid et al., 2018; Ayed et al., 2021). Osmoprotectants, such as proline, can be triggered by drought stress to help detoxify oxidative stress and maintain osmotic adjustments (Shrestha et al., 2022). Plants may utilize either individual mechanisms or a combination of the above mechanisms in response to drought stress.

Common or bread wheat (*Triticum aestivum* L., genome BBAADD, 2n = 6x = 42) is a major cereal crop worldwide. It is a key source of calories and proteins for the global population (Food and Agricultural Organization FAO, 2023; Langridge et al., 2022). Wheat wild relatives and landraces may have developed specific adaptations to their local environments and therefore offer valuable genetic resources for introducing drought tolerance into breeding programs (Aberkane et al., 2020; Ahmadi et al., 2018; Leigh et al., 2022; Lopes et al., 2015; Nevo and Chen, 2010; Peng et al., 2011). Drought stress affects the morphological, physiological, and biochemical traits of wheat plants (Ayed et al., 2021; Pour-Aboughadareh et al., 2017; Suneja et al., 2017). Previous studies have examined drought adaptation traits in different wheat genotypes, which vary from the field to the gene level (Farrell et al., 2017; Lopes et al., 2014; Ma et al., 2017; Nevo and Chen, 2010; Reynolds et al., 2007; Wen et al., 2023). Stress tolerance mechanisms such as stomatal regulation, maintenance of water content, and proline production have mostly been studied in domesticated wheat (Ahmadi et al., 2018; Mwadzingeni, et al., 2016; Saghour el idrissi et al., 2023). Additionally, some wild species, such as *T. dicoccoides*, *T. boeoticum*, and *T. urartu*, have been investigated for their drought stress physiology (Peleg et al., 2009; Pour-Aboughadareh et al., 2017; Suneja et al., 2017). However, linkages between their drought adaptation mechanisms and yield components have not been fully explored. Furthermore, despite its significant potential for wheat breeding, *T. araraticum* (GGA<sup>1</sup>A<sup>5</sup>), a wild tetraploid, has not been widely studied (Badaeva et al., 2022). To the best of our knowledge, very few studies have addressed the abiotic stress physiology of *T. araraticum* (GGA<sup>1</sup>A<sup>5</sup>) (Badaeva et al., 2022; Sultan et al., 2012). Moreover, few studies have addressed the effects of drought stress caused by repetitive drought events during the stages of stem elongation and anthesis in wild wheats and landraces (Bapela et al., 2022; Nevo and Chen, 2010). Therefore, an understanding of stress-adaptive changes during these two stages and the associated mechanisms to maintain stable yield under drought stress is crucial for advancing wheat breeding programs.

The effects of drought stress on a diverse panel of *Triticum* taxa have not been fully characterized, and the underlying mechanisms of drought stress tolerance have yet to be elucidated. It is therefore necessary to explore a wide range of genetic diversity to gain a better understanding of individual drought tolerance mechanisms (Bao et al., 2023; Lopes et al., 2014, 2015). Therefore, the objective of this study was to investigate the effects of drought stress on morphophysiological traits associated with biochemical markers in different genotypes of the *Triticum* genus. Accordingly, we conducted a pot experiment under greenhouse conditions with drought simulations introduced during the stem elongation and anthesis developmental stages under two different treatments: well-watered and drought stress conditions. We used a diverse panel of wild wheat, landraces, and modern cultivars belonging to three different ploidy levels and eight different taxa. The following hypotheses were formulated:

- (1) Drought tolerance mechanisms for osmotic and stomatal regulation counteracting oxidative stress are correlated between the stem elongation and anthesis stages and affect grain yield loss;
- (2) Compared with modern cultivars, wild wheat plants exhibit greater drought tolerance.

An experiment to verify these hypotheses can provide valuable input for developing drought-tolerant cultivars in wheat breeding programs by incorporating different drought tolerance mechanisms.

## 2. Materials and methods

### 2.1. Plant material

A total of seventeen genotypes were used in this experiment, comprising eleven wild wheat samples from four taxa, three

domesticated landraces from three taxa, and three modern cultivars from two taxa (Table 1). The wheat taxa were categorized according to the classification system outlined in a previous study (Zeibig et al., 2024). The accessions were selected on the basis of the results of previous field experiments. The wild wheat genotypes and domesticated landraces were sourced from the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany, and from Çukurova University, Turkey. For comparison purposes, two modern bread wheat cultivars, *T. aestivum* cv. Apostel (I.G. Pflanzenzucht GmbH) and cv. Nordkap (SAATEN UNION), and one modern durum wheat cultivar, *T. durum* cv. Sambadur (Hauptsaaften für die Rheinprovinz GmbH), were included in the study.

**Table 1**

Classification of genotypes into taxa, biological status, and ploidy levels, with their countries of origin.

Taxon	Biological status	Genome	Genotype	Country of origin and seed collection site
<b>Diploid, 2 n = 2x = 14</b>				
<i>T. urartu</i> Thumanjan ex Gandilyan.	Wild	A <sup>u</sup>	G45	Syria: 3 km northwest of Salah; on the road to Sousan
<i>T. boeoticum</i> Boiss.	Wild	A <sup>b</sup>	G27	Turkey: 52.5 km northeast of Urfa, near Hilvan
<i>T. boeoticum</i> Boiss.	Wild	A <sup>b</sup>	G33	Turkey: 69 km northwest of Kirsehir
<i>T. monococcum</i> subsp. <i>sinskajae</i> (Filat. & Kurkiew)	Landrace	A <sup>m</sup>	G89	Turkey
<b>Tetraploid, 2 n = 4x = 28</b>				
<i>T. dicoccoides</i> (Körn. ex Asch. & Graebn.) Schweinf.	Wild	BA	G90	Turkey: 9.3 km southeast from Ergani toward Diyarbakir
<i>T. dicoccoides</i> (Körn. ex Asch. & Graebn.) Schweinf.	Wild	BA	G242	Israel: Tabigha
<i>T. dicoccoides</i> (Körn. ex Asch. & Graebn.) Schweinf.	Wild	BA	G248	Iraq: northern slope of Jabal Sinjar north of Kursi
<i>T. durum</i> Desf.	Modern cultivar	BA	G41	cv. Sambadur (Hauptsaaften), Germany
<i>T. araraticum</i> Jakubz.	Wild	GA <sup>t</sup>	G161	Iraq: 10.6 km east-northeast from Koi Sanjaq toward Ranya
<i>T. araraticum</i> Jakubz.	Wild	GA <sup>t</sup>	G189	Iran
<i>T. araraticum</i> Jakubz.	Wild	GA <sup>t</sup>	G205	Iraq: 33.2 km west from Rowanduz toward Shaqlawa
<i>T. araraticum</i> Jakubz.	Wild	GA <sup>t</sup>	G221	Iraq: 4.4 km northwest from Amadiyah, Mazorka Gorge
<i>T. araraticum</i> Jakubz.	Wild	GA <sup>t</sup>	G222	Iraq: 19.3 km south from Sulaymaniyah toward Qara Dagh
<b>Hexaploid, 2 n = 6x = 42</b>				
<i>T. aestivum</i> L.	Landrace	BAD	G10	Afghanistan: Herat and Palpiri
<i>T. aestivum</i> L.	Modern cultivar	BAD	G134	cv. Apostel (I.G. Pflanzenzucht), Germany
<i>T. aestivum</i> L.	Modern cultivar	BAD	G181	cv. Nordkap (SAATEN UNION), Germany
<i>T. spelta</i> L.	Landrace	BAD	G109	Iran: Noach, 18 km from Shahr-Kord

## 2.2. Experimental conditions and treatments

The experiment was conducted during the 2022 winter wheat growing season (from October 2022 to May 2023) at Justus-Liebig-University, Germany, under controlled greenhouse conditions. A minimum photosynthetically active photon flux density (PPFD) of 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  during the light period was ensured by natural and artificial light in the greenhouse. Eighteen uniformly sized seeds of each genotype were sown in Quickpot trays on October 4, 2022. After 48 hours of stratification at 5 °C, the seedlings were germinated in a greenhouse under light/dark conditions of 10 h/14 h and day/night temperatures of 15 °C/10 °C. After germination, the plants continued to grow until they reached the 2–3 leaf stage (Z12; Zadoks et al., 1974) under light/dark conditions of 8 h/16 h and day/night temperatures of 10 °C/5 °C and a relative humidity of .55–60 %. The plants underwent vernalization under natural conditions inside a netted chamber for two months and were subsequently allowed to harden under greenhouse conditions (light/dark conditions of 8 h/16 h and day/night temperatures of 10 °C/5 °C) for two weeks. The plants were then transplanted to 5 L plastic pots filled with N-type soil substrate (Hawita Gruppe GmbH, Germany) for six replications in total, with two plants per pot in three replications and one plant per pot in the remaining three replications. A randomized complete block design was used with two treatments: well-watered conditions and drought stress conditions. After one week, the following growth conditions were used: a light/dark period of 10 h/14 h and day/night temperatures of 15 °C/10 °C to promote gradual acclimatization over one week, followed by a light/dark period of 16 h/8 h, day/night temperatures of 21 °C/16 °C, and 55–60 % relative humidity maintained until crop maturity.

The soil moisture content was measured with an HH2 soil moisture meter (Delta-T Devices Ltd., version 4.2, Cambridge, UK), and the volumetric water content was assessed to provide a reference for the pot soil water-holding capacity (PWHC) in a 5 L pot. Measurements of moisture content were taken every two to three days from the time of transplanting until the plants reached physiological maturity (Figure S1). Well-watered plants received regular irrigation to maintain the soil moisture content at 80–90 % of the PWHC throughout the growth period. Drought stress treatment during the stem elongation stage commenced at Z25 (Zadoks et al., 1974), with cessation of irrigation for 16 days until the average soil moisture reached a level below 15 % of the PWHC. After the physiological parameters were measured and a fresh sample was harvested during the stem elongation stage, 16 days after the drought stress treatment, the plants were rewatered to restore the soil moisture to 80–90 % of the PWHC, which was consistent with that of the well-watered treatment group. A second simulation was implemented targeting the anthesis stage, specifically for individual genotypes at Z49, to induce drought stress during the anthesis stage (Z60 to Z73; Zadoks et al., 1974) until the average soil moisture decreased to 18 % of the PWHC. The same potted plants were subjected to drought stress at both the stem elongation and anthesis stages. Following the collection of physiological data and the harvest of fresh samples during the anthesis stage, the plants were rewatered to maintain the soil moisture at 80–90 % PWHC until physiological maturity was attained (Z93).

## 2.3. Harvest of fresh samples

The plants were harvested fresh during the stem elongation stage after 16 days of drought stress treatment. For all the genotypes, one plant from each of the first three replications that contained two plants per pot was harvested from both the well-watered and drought treatments and flash frozen in liquid nitrogen. Fresh harvesting during drought treatment at the anthesis stage was performed at Z73 (Zadoks et al., 1974) for individual genotypes when the average soil moisture decreased to 18 % of the PWHC. Two to three leaves below the flag leaf were harvested at the anthesis stage for biochemical analysis.

Immediately after the fresh harvest, all samples from both the stem elongation and anthesis stages were stored at  $-80^{\circ}\text{C}$  for biochemical analysis. All the study parameters were measured during drought episodes for both the well-watered and drought stress treatments.

#### 2.4. Agro-morphological traits

During the stem elongation stage, shoot length (SHL) was measured from the soil surface to the tip of the longest, fully expanded leaf, and total tillers (TT) were counted 16 days after the drought stress treatment prior to harvesting the fresh samples. Phenological data, such as days to heading (DTH) and days to anthesis (DTA), were recorded for individual plants at Z50 and Z60 (Zadoks et al., 1974), respectively. Days to maturity (DTM) were recorded when the main spike turned fully yellow at Z90. The phenological days were recorded with the seed sowing date to provide reference values for the respective phenological stages. Plant height (PH) was measured from the soil surface to the top of the spike (excluding the awns). Spike length (SL) was measured from the base to the top of the spike (excluding the awns). The peduncle exertion length (PEL) was measured from the base of the flag leaf sheath to the base of the spike. PH, SL, and PEL were measured after anthesis. The number of effective tillers (ET) was counted after the plants reached physiological maturity. The aboveground biomass weight (BM) per plant was measured after the sample was oven-dried at  $30^{\circ}\text{C}$  to reach a constant weight. The spikes were then threshed in a coffee mill, after which the grain yield (GY) per plant and thousand kernel weight (TKW) were measured. For each plant, 50 seeds were counted three times, and the TKW was calculated with reference to the average weight of 50 seeds. The grains were measured for TKW, and the number of grains (GN) per plant was determined via a Contador Seed Counter (Pfeuffer GmbH, Kitzingen, Germany). The harvest index (HI) for each plant was calculated as a percentage by dividing the GY by the BM per plant.

#### 2.5. Physiological traits

Spectral reflectance measurements (vegetation indices) were recorded using a Polyphen RP 410 portable instrument (Photon Systems Instruments, Drasov, Czech Republic). The following indices were calculated: normalized difference vegetation index (NDVI) =  $(R780 - R630)/(R780 + R630)$ ; Lichtenthaler index 2 (Lic2) =  $R440/R690$  (Begum et al., 2020); anthocyanin reflectance indices (ARI2) =  $R790/(1/R550 - 1/R700)$  (Gitelson and Merzlyak, 1997); carotenoid reflectance index 2 (CRI2) =  $1/R510 - 1/R700$  (Gitelson et al., 2003); and modified chlorophyll absorption reflectance index (MCARI1) =  $1.2 \times [2.5 \times (R790 - R670) - 1.3 \times (R790 - R550)]$  (Daughtry et al., 2000). A portable handheld LI-600 porometer system integrated with a fluorometer (LI-COR, Lincoln, Nebraska, USA) was used to measure stomatal conductance (gsw) and photosystem II efficiency (PhiPS2). All of these measurements were performed before the harvest of fresh samples during both the stem elongation and anthesis stages under drought stress.

The water status of the leaves was measured by the relative water content (RWC). To measure RWC, a mid-leaf section (approximately 4–5 cm) was cut from the leaf, and the fresh weight (FW) was immediately recorded. The leaf section was subsequently dipped in a 15 ml Falcon tube filled with 10 ml of deionized water for 24 h at room temperature. The leaf sample was removed from the tube, and the turgid weight (TW) was measured immediately after excess water was removed with tissue paper. Dry weight (DW) was recorded after the leaf sections were oven-dried at  $80^{\circ}\text{C}$  for 48 h. RWC was calculated as  $\text{RWC} = (\text{FW} - \text{DW})/(\text{TW} - \text{DW}) \times 100$ .

#### 2.6. Biochemical traits

The proline content was analyzed applying the method developed by Bates et al. (1973), with slight modifications. Sulfosalicylic acid (3 %

w/v) and ninhydrin reagents (2.5 g of ninhydrin in 60 ml of glacial acetic acid and 40 ml of 6 M phosphoric acid) were used to determine the proline content. In brief, the samples were ground in liquid nitrogen, and 50 mg of the chilled sample was placed in a 2 ml microcentrifuge tube. Next, 1.5 ml of 3 % sulfosalicylic acid was added, and the mixture was vortexed vigorously before being centrifuged at  $12,000 \times g$  for 10 min. Then, 500  $\mu\text{l}$  of the supernatant was added to a 10 ml glass test tube, 500  $\mu\text{l}$  of acetic acid and 500  $\mu\text{l}$  of ninhydrin reagents were added, and the mixture was vortexed thoroughly. The mixture was subsequently incubated at  $100^{\circ}\text{C}$  for one hour. The reaction was immediately stopped by placing the tube on ice, and 1.5 ml of pure toluene was added, vortexed, and left at room temperature for 30 min. The absorbance of the chromatophore was measured at 520 nm via a microplate reader (Infinite 200 Pro, Tecan, Groedig, Austria). The sample proline content was calculated using the standard curve approach and expressed in micrograms per gram of fresh weight ( $\mu\text{g g}^{-1}$  FW).

Lipid peroxidation was determined according to the malondialdehyde (MDA) concentration using the thiobarbituric acid (TBA) method described by Ali et al. (2019). In brief, 100 mg of fresh tissue was homogenized in 1.5 ml of 0.1 % (w/v) trichloroacetic acid (TCA) and centrifuged at  $14,000 \times g$  for 15 min at  $4^{\circ}\text{C}$ . The reaction mixture (RSI) was prepared by dissolving 0.01 % (v/v) 2,6-di-tert-butyl-4-methylphenol (BHT) in 20 % TCA (v/v), and the reaction mixture (RSII) was prepared by dissolving 0.65 % TBA (w/v) in warm RSI solution. Next, 500  $\mu\text{l}$  of plant extract was added to 500  $\mu\text{l}$  of RSI and 500  $\mu\text{l}$  of RSII in separate 15 ml Falcon tubes. The reaction mixture was incubated at  $95^{\circ}\text{C}$  for 30 min. After incubation, the reaction was stopped by placing the tube on ice for 5 min, and the reaction mixture was then transferred to a 1.5 ml centrifuge tube and centrifuged at  $8000 \times g$  for 10 min at  $4^{\circ}\text{C}$ . The absorbance was measured at 440, 532, and 600 nm using a microplate reader (Infinite 200 Pro, Tecan, Groedig, Austria).

#### 2.7. Data analysis

To rank the genotypes for drought tolerance, a relative value was calculated for each genotype using the method described by Bouslama and Schapaugh (1984), with slight modifications. Specifically, it was calculated for each genotype as the ratio of the drought value divided by the average well-watered value for each studied trait. Relative values are useful for comparing highly variable genotypes across different taxa. The genotypes were sorted and arranged in ascending order, from low to high relative values in the bar graphs. The statistical analysis was performed in R Studio with R version 4.2.0 ([www.r-project.org](http://www.r-project.org)). Analysis of variance (ANOVA) was performed via a mixed linear model in the nlme package (<https://cran.r-project.org/web/packages/nlme/index.html>), with genotype and treatment considered fixed factors and replication by treatment interaction considered a random effect. Multiple comparison tests were performed via the emmeans package (<https://cran.r-project.org/web/packages/emmeans/index.html>). The correlation plots were generated via multi-environment trial analysis (<https://cran.r-project.org/web/packages/metan/index.html>), (<https://cran.r-project.org/web/packages/ggpubr/index.html>) and the corrplot (<https://cran.r-project.org/web/packages/corrplot/index.html>) package in R to determine Pearson's correlation. Principal component analysis (PCA) was conducted using FactoMineR (<https://cran.r-project.org/web/packages/FactoMineR/index.html>) and Factoextra (<https://cran.r-project.org/web/packages/factoextra/index.html>). Figures were generated with ggplot2 (<https://cran.r-project.org/web/packages/ggplot2/index.html>) and Microsoft Excel.

### 3. Results

#### 3.1. Changes in physiological traits

At both the stem elongation and anthesis stages and across all the genotypes, gsw and RWC decreased significantly due to drought stress

**Table 2**

Descriptive statistics and analysis of variance (ANOVA) for the physiological and biochemical traits of 17 genotypes from the *Triticum* genus measured at the stem elongation and anthesis stages.

Traits	Mean (Standard Deviation)		ANOVA		
	Well-watered	Drought	Treatment	Genotype	Treatment × Genotype
<b>Stem elongation stage</b>					
Relative water content (%)	91.7 (3.3)	61.9 (15.1)	**	*	**
Proline (μg/g, FW)	56.1 (15.5)	3998.6 (1973.9)	**	****	****
Malondialdehyde (nmol/ml/g, FW)	16.4 (5.4)	25.2 (5.4)	**	****	*
gsw (mmol m <sup>-2</sup> s <sup>-1</sup> )	416.2 (155.7)	52.5 (77.1)	****	****	****
PhiPS2	0.64 (0.09)	0.65 (0.08)	ns	**	ns
NDVI	0.60 (0.03)	0.59 (0.04)	ns	****	ns
Lic2	0.97 (0.16)	1.00 (0.17)	ns	**	ns
CR12	1.48 (0.85)	1.18 (0.80)	ns	**	ns
ARI2	-0.21 (0.17)	-0.25 (0.19)	ns	**	ns
MCARI1	2.01 (2.21)	2.35 (2.44)	ns	*	ns
<b>Anthesis Stage</b>					
Relative water content (%)	89.1 (6.5)	65.0 (15.5)	*	*	*
Proline (μg/g, FW)	53.7 (10.6)	1856.5 (1578.6)	ns	***	***
Malondialdehyde (nmol/ml/g, FW)	10.1 (2.5)	16.1 (3.5)	**	****	ns
gsw (mmol m <sup>-2</sup> s <sup>-1</sup> )	239.6 (244.5)	52.1(96.9)	****	****	****
PhiPS2	0.64 (0.09)	0.66(0.10)	ns	**	*
NDVI	0.57 (0.05)	0.57(0.05)	ns	****	ns
Lic2	0.89 (0.08)	0.91(0.11)	*	****	ns
CR12	1.71 (0.56)	1.34(0.67)	**	****	ns
ARI2	-0.11 (0.08)	-0.14(0.12)	ns	****	ns
MCARI1	0.69 (0.05)	0.73(0.07)	**	****	**

Note: Mean values across all genotypes are shown. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . ns = nonsignificant, FW = fresh weight, gsw = stomatal conductance, PhiPS2 = photosystem II efficiency, ARI2 = anthocyanin reflectance index 2, CR12 = carotenoid reflectance index 2, Lic2 = Lichtenthaler index 2, MCARI1 = modified chlorophyll absorption reflectance index 1, NDVI = normalized difference vegetation index.

(Table 2). On average, gsw and RWC decreased by 87 % and 32 %, respectively, during the stem elongation stage and by 78 % and 27 %, respectively, during the anthesis stage. However, the Lic2 and MCARI1 vegetative indices increased significantly across all the genotypes due to drought stress, on average, by 25 % and 5 %, respectively. At the stem elongation stage, CR12 decreased significantly by 22 %, but no such effect was observed for the vegetation indices (Table 2). All the genotypes showed significant gsw responses to the drought stress treatment during the stem elongation stage (Fig. 1A). However, during the anthesis stage, not all of them revealed significant gsw responses to drought stress (Fig. 1B). Whereas drought stress induced significant reductions in gsw in *T. boeoticum* (G33, G27), *T. urartu* (G45), and *T. dicoccoides* (G242), reductions in gsw were lower in *T. araraticum* (G161 and G189).

Under drought stress, the RWC decreased in all the genotypes during both the stem elongation and anthesis stages. However, the decrease was not significant in some of the genotypes (Figs. 1C and 1D). The lowest decrease in RWC was observed for *T. monococcum* subsp. *sinskajae* (G89), *T. urartu* (G45), *T. araraticum* (G205), and *T. boeoticum* (G33) during the stem elongation stage (Fig. 1C). During the anthesis stage, decreases in RWC were lowest for *T. boeoticum* (G27), *T. spelta* (G109), *T. dicoccoides* (G242), *T. urartu* (G45), and *T. araraticum* (G205) (Fig. 1D). During both stages, these genotypes showed average relative values  $>0.8$ . The genotype effect was significant for all physiological traits, such as RWC, gsw, PhiPS2, NDVI, Lic2, ARI2, CR12, and MCARI1, during both the stem elongation and anthesis stages (Table 2). The treatment × genotype effect was highly significant for RWC and gsw during the stem elongation stage and was significant for various physiological traits, such as RWC, gsw, PhiPS2, and MCARI1, during the anthesis stage (Table 2).

### 3.2. Changes in malondialdehyde content

Across all the genotypes, the MDA content increased significantly by 53 %, on average, during the stem elongation stage and by 58 % during the anthesis stage due to drought stress (Table 2). The effect of genotype on the MDA content was highly significant during both stages. However, the treatment × genotype interaction effect was significant only during the stem elongation stage. The magnitude of the increase in MDA was lower in *T. aestivum*, cv. Nordkap (G181), *T. aestivum*, cv. Apostel

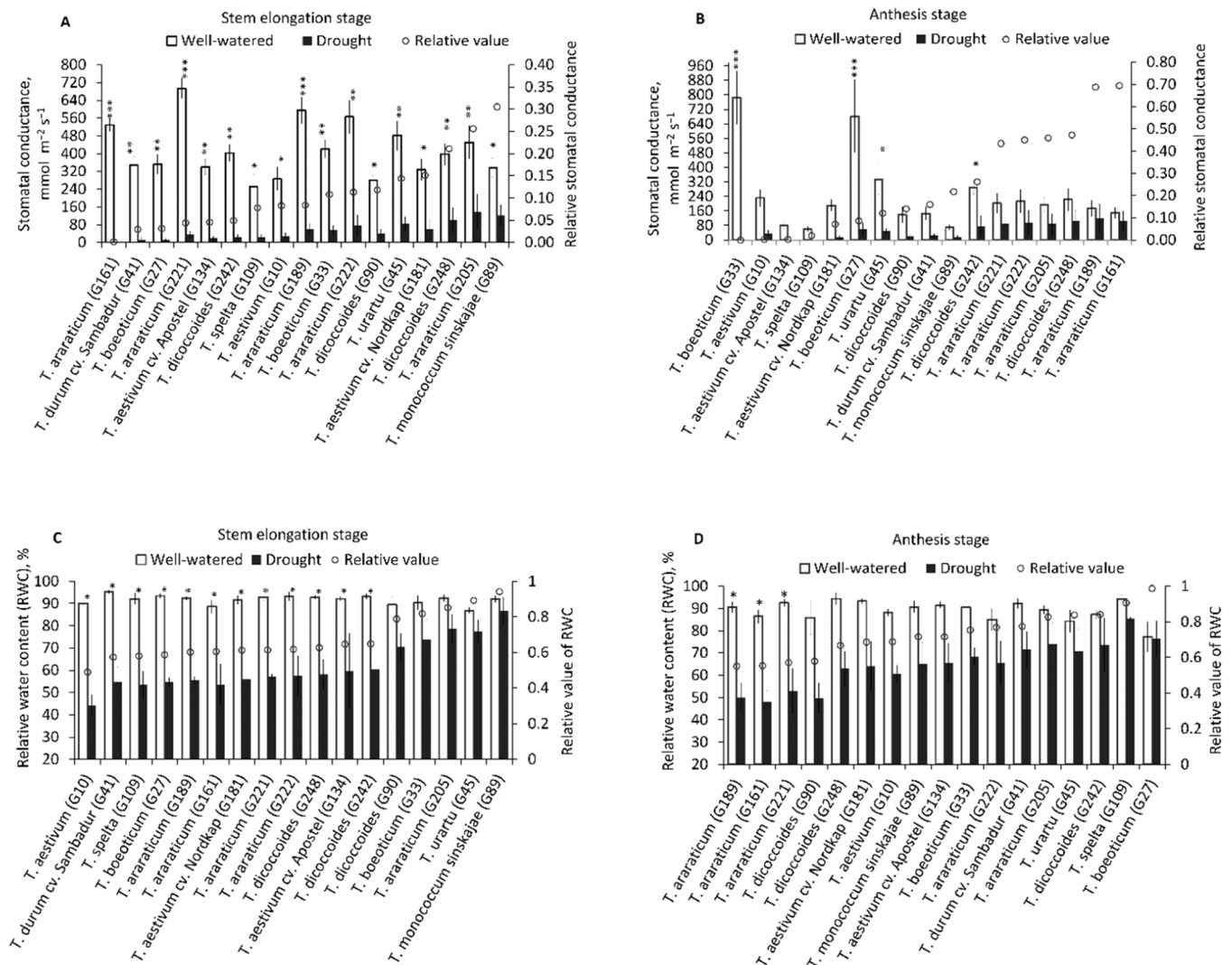
(G134), and *T. araraticum* (G205), but it increased significantly in *T. durum*, cv. Sambadur (G41) and *T. araraticum* (G221) during the stem elongation stage (Fig. 2A). Similarly, during the anthesis stage, the magnitude of the increase in MDA was lower in *T. araraticum* (G189, G221, and G222), *T. aestivum*, cv. Nordkap (G181), *T. dicoccoides* (G242), and *T. spelta* (G109), whose relative values were  $<1.5$  in the drought scenario (Fig. 2B). The magnitude of the increase in MDA was significantly greater in *T. boeoticum* (G33), *T. durum*, cv. Sambadur (G41), and *T. dicoccoides* (G90) than in the other genotypes.

### 3.3. Changes in proline content

During both the stem elongation and anthesis stages, the average proline concentration increased across all the genotypes due to drought stress (Table 2). During the stem elongation stage, proline increased significantly approximately 71-fold, and during the anthesis stage, it increased approximately 35-fold, which was close to significance ( $p = 0.08$ ; not shown). Furthermore, the effects of genotype and the treatment × genotype interaction were highly significant during both stages. During the stem elongation stage (Fig. 2C), most of the genotypes apart from *T. monococcum* subsp. *sinskajae* (G89), *T. araraticum* (G205), *T. urartu* (G45), *T. boeoticum* (G33) and *T. dicoccoides* (G90) presented significant increases in proline content induced by drought stress. In contrast, during the anthesis stage (Fig. 2D), only *T. dicoccoides* (G90) showed a significant increase (approximately 51-fold) in proline content. During the stem elongation stage, *T. araraticum* (G221) revealed the greatest increase in proline content (approximately 125-fold).

### 3.4. Changes in crop phenology, morphology, and yield-related traits

Drought stress significantly affected the onset of phenological stages, such as DTH and DTA, whose values were averaged across all the genotypes (Table 3). However, not all the genotypes evidenced significant phenological acceleration. Significant DTA enhancement only occurred in some genotypes, such as *T. urartu* (G45), *T. dicoccoides* (G242), *T. monococcum* subsp. *sinskajae* (G89), *T. boeoticum* (G33), and *T. dicoccoides* (G90) (Figure S2A), whereas significant DTM enhancement was observed only in *T. dicoccoides* (G242 and G248) (Figure S2B).

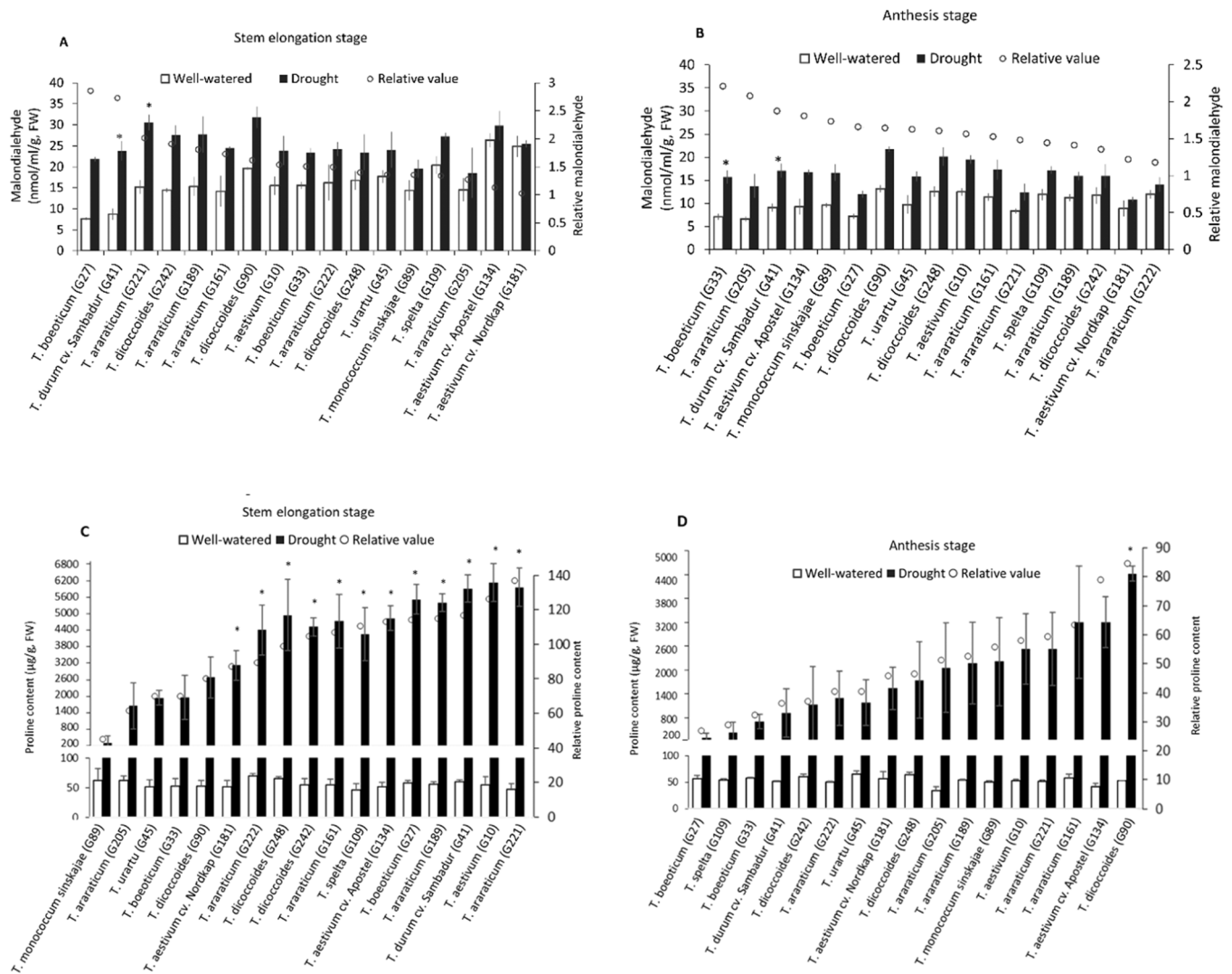


**Fig. 1.** Stomatal conductance at the stem elongation stage (A) and the anthesis stage (B) and relative water content at the stem elongation stage (C) and the anthesis stage (D). The unshaded circles indicate the mean relative values (drought/well-watered) on the secondary vertical axis, and the genotypes are arranged in ascending order from low relative values (left) to high relative values (right). The white and black vertical bars indicate the mean values under well-watered and drought conditions, respectively, on the primary vertical axis. The data are the average values of five replicates  $\pm$  standard errors for stomatal conductance at the stem elongation stage, the average values of six replicates  $\pm$  standard errors for stomatal conductance at the anthesis stage, and the average values of three replicates  $\pm$  standard errors for relative water content at both stages. Asterisks above the vertical bar indicate significant differences between the well-watered and drought conditions (treatment effect) within the same genotype according to pairwise comparisons (\* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$ ).

Drought stress had a severe effect on yield-related traits, such as GY, BM, and GN, which were reduced by 51 %, 49 %, and 46 %, respectively, across all the genotypes (Table 3). However, not all the genotypes exhibited significant decreases in GY, BM, and GN. The decrease in GY was lower for *T. monococcum* subsp. *sinkajae* (G89), *T. boeoticum* (G27), and *T. dicoccoides* (G242), with a relative value  $>0.6$ , and the greatest decrease was observed for *T. aestivum* (G10) (Fig. 3A). *T. monococcum* subsp. *sinkajae* (G89) and *T. aestivum* (G10) exhibited the lowest and greatest decreases in BM, respectively (Fig. 3B). *T. monococcum* subsp. *sinkajae* (G89) showed no GN-related response to drought stress (Fig. 3C). The average TKW and HI values obtained across all the genotypes revealed that their treatment effects were nonsignificant. However, the treatment  $\times$  genotype effect was highly significant for both of these traits (Table 3). Drought stress induced significant decreases in TKW in *T. monococcum* subsp. *sinkajae* (G89) and *T. aestivum* (G10). In contrast, some genotypes under drought stress, notably *T. dicoccoides* (G242 and G248), *T. aestivum* cv. Nordkap (G181), *T. boeoticum* (G27 and G33), and *T. urartu* (G45), presented no response to TKW, with relative values of approximately 1. Interestingly, drought

stress induced a significant increase in the HI for *T. dicoccoides* (G242) (Fig. 3E), whereas the HI for *T. aestivum* cv. Apostel (G134) decreased significantly.

The average values across all the genotypes indicated that morphological traits, such as PH, ET, SL, and PEL, were significantly negatively affected by drought stress (Table 3). PH decreased significantly in the genotypes belonging to all four wild taxa and the three landrace taxa but not in the three modern cultivars (Fig. 3F). Genotype effects and treatment  $\times$  genotype interaction effects were highly significant for all phenological, morphological, and yield-related traits apart from ET, DTH, and DTA, which were significant for genotype and treatment effects (Table 3). We also investigated drought stress responses related to TT and SHL, which were obtained as average values for all the genotypes during the stem elongation stage. Both of these traits showed significant treatment and genotype effects (Table 3). However, the treatment  $\times$  genotype effect was significant only for SHL.



**Fig. 2.** Malondialdehyde content at the stem elongation stage (A) and the anthesis stage (B); proline content at the stem elongation stage (C) and the anthesis stage (D). The unshaded circles indicate the mean relative values (drought/control) on the secondary vertical axis, and the genotypes are arranged from low relative values (left) to high relative values (right) for proline and from high relative values (left) to low relative values (right) for malondialdehyde. The white and black vertical bars indicate the mean values under well-watered and drought conditions, respectively, on the primary vertical axis. The data are the average values of three replicates  $\pm$  standard errors. Asterisks above the vertical bar indicate significant differences between the well-watered and drought conditions (treatment effect) within the same genotype according to pairwise comparisons (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ).

### 3.5. Statuswise responses to drought stress for various traits during the stem elongation and anthesis stages

We investigated the responses of the plants to drought stress according to their biological status for morphological, physiological, biochemical, and yield-related traits measured at the stem elongation (Table S1) and anthesis stages (Table S2). At the stem elongation stage, gsw and RWC significantly decreased in the wild, landrace, and modern cultivar groups. However, at the anthesis stage, these parameters decreased significantly only in the wild-type group. At the stem elongation stage, proline increased significantly in all the status groups, but there was no significant difference at the anthesis stage. At the stem elongation stage, the SHL decreased significantly in the wild-type group under drought stress, whereas the TT was affected in all the status groups at this stage. However, the ET measured at the maturity stage was not significant for any of the status groups, whereas the PH was significant for both the wild and landrace groups. Under severe drought stress applied at two different stages, we observed significant decreases in yield-related traits, such as GY, BM, and GN, across all the status groups. Among the status groups, only modern cultivars showed a

significant HI response (Table S2).

### 3.6. Correlations between the stem elongation and anthesis stages for biochemical and physiological traits

We investigated correlations between the stem elongation and anthesis stages related to biochemical and physiological traits (Fig. 4 and Table S3). A weak correlation between the stem elongation and anthesis stages was observed for physiological traits, such as gsw and RWC, and for biochemical traits, such as proline and MDA (Fig. 4). The subsequent analysis revealed positive correlations for gsw ( $R^2 = 0.18$ ,  $p < 0.001$ ), RWC ( $R^2 = 0.41$ ,  $p < 0.001$ ), proline ( $R^2 = 0.31$ ,  $p < 0.001$ ) and MDA ( $R^2 = 0.43$ ,  $p < 0.001$ ), indicating a weak linear relationship and a highly significant association between the two stages. There was more variability in these traits across the two stages for wild taxa than for landraces and modern cultivars. However, no associations were found between the stem elongation and anthesis stages for vegetative indices, such as the NDVI, Lic2, MCARI2, CRI2, and ARI2 (Table S3).

**Table 3**

Descriptive statistics and analysis of the morphological, phenological, and yield traits of 17 genotypes in the *Triticum* genus.

Traits	Mean (Standard deviation)		ANOVA		
	Well-watered	Drought	Treatment	Genotype	Treatment × Genotype
Total tillers: stem elongation stage	13 (4.3)	8 (3.3)	***	****	ns
Shoot length (cm): stem elongation stage	69.4 (15.0)	56.9 (11.2)	****	****	****
Days to heading	153 (6.8)	150 (7.2)	**	****	ns
Days to anthesis	159 (6.4)	156 (6.2)	**	****	ns
Days to maturity	190 (5.8)	188 (7.8)	ns	****	**
Plant height (cm)	119.1 (23.7)	90.3 (15.6)	****	****	****
Spike length (cm)	10.1 (2.4)	9.4 (2.3)	**	****	****
Effective tillers	17 (8.3)	14 (8.9)	*	****	ns
Peduncle exertion length (cm)	26.4 (10.8)	16.8 (9.1)	**	****	***
Above ground biomass (g)	37.2 (14.8)	18.9 (7.0)	****	****	****
Grain yield (gm)	12.5 (8.3)	6.1 (3.4)	**	****	****
Grain number	450 (232)	242 (121)	**	****	****
Thousand kernel weight (g)	29.8 (13.0)	28.3 (12.6)	ns	****	**
Harvest index (%)	32.1 (11.0)	31.5 (9.1)	ns	****	****

Note: Mean values of all genotypes are shown. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  \*\*\*\* $p < 0.0001$ , ns = nonsignificant.

### 3.7. Associations among the measured traits

We investigated the associations among all the measured traits during the stem elongation stage (Fig. 5) and anthesis stage (Fig. 6) under both well-watered and drought conditions.

#### 3.7.1. Correlations of physiological and biochemical traits with yield-related traits

During the stem elongation stage, none of the physiological or biochemical traits, apart from the NDVI, were associated with yield-related traits under either well-watered or drought stress conditions (Figs. 5A and 5B).

During the anthesis stage, gsw was significantly and negatively correlated with GY and BM under drought stress conditions (Fig. 6B). Similarly, the NDVI was negatively correlated with BM, GY, and TKW under these conditions, but no such correlation occurred under well-watered conditions. Lic2 was significantly and positively correlated with GY and HI under both well-watered and drought stress conditions. In contrast, MCARI1, CRI2, and ARI2 were significantly and negatively correlated with several yield-related traits. For example, MCARI1 was negatively correlated with HI and TKW under well-watered conditions (Fig. 6A) and with BM, GY, HI, and TKW under drought conditions (Fig. 6B). CRI2 was negatively correlated with HI, GY, and TKW under both well-watered and drought conditions. However, other physiological and biochemical traits, such as proline and MDA contents and RWC, were not associated with yield-related traits under either well-watered or drought conditions during the anthesis stage (Figs. 6A and 6B).

#### 3.7.2. Correlations of physiological and biochemical traits

Notably, during the stem elongation stage (Figs. 5A and 5B), gsw was significantly positively correlated with RWC and significantly negatively correlated with the MDA and proline contents under drought stress conditions. However, no such associations were observed under well-watered conditions. Similarly, under drought stress, ARI2 was significantly negatively correlated with the MDA and proline contents but significantly positively correlated with gsw and RWC. These associations were not detected under well-watered conditions. Moreover, no associations were detected between proline and MDA contents during the stem elongation stage under either well-watered or drought conditions.

During the anthesis stage (Figs. 6A and 6B), gsw was significantly negatively correlated with MDA and RWC under well-watered conditions but not under drought conditions. Similarly, under drought conditions, the proline content was significantly negatively correlated with RWC but significantly positively correlated with MDA. Such associations were not observed under well-watered conditions. Under drought conditions, MCARI1 was significantly and positively correlated with gsw but negatively correlated with RWC. However, under well-watered conditions, no associations were detected between gsw or RWC and any of the vegetative indices. In addition, biochemical traits, such as proline and MDA contents, did not exhibit any correlations with vegetative indices under either well-watered or drought conditions.

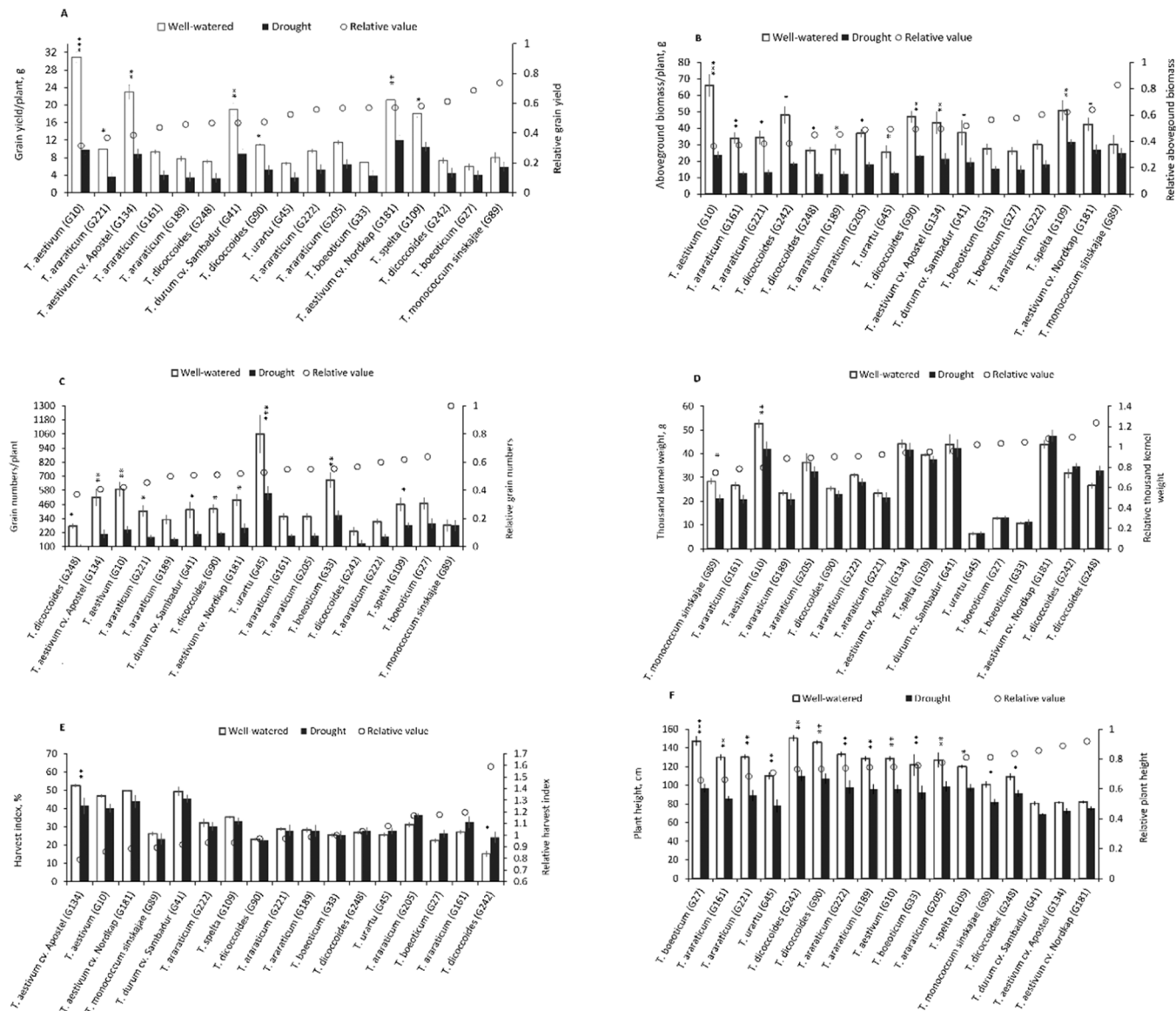
#### 3.7.3. Correlations of morphological and phenological traits with yield-related traits

TT was significantly negatively associated with TKW at the stem elongation stage under drought stress (Fig. 5B), which was consistent with the finding of a significant negative correlation between ET and TKW at the anthesis stage (Fig. 6B). However, SHL was significantly positively associated with TKW at the stem elongation stage, but no such association was observed between PH and TKW under drought stress.

Notably, SL was positively associated with GY under drought stress but not under well-watered conditions. Similarly, GN was negatively correlated with TKW under drought stress but not under well-watered conditions. PH was negatively correlated with HI under both well-watered and drought stress conditions. PEL was negatively correlated with yield-related traits, such as GY, HI, and TKW, under both well-watered and drought stress conditions. Under drought stress conditions, DTM was positively correlated with GY and BM; however, this association was not detected under well-watered conditions. As we expected, GY was significantly and positively correlated with HI, TKW, and BM under both well-watered and drought stress conditions.

### 3.8. Principal component analysis

A PCA was performed to investigate associations among various traits and the distributions and associations of the genotypes with these traits. The PCA results revealed that during the stem elongation stage, PC1 explained 31 % of the variation, and PC2 explained 22.3 % of the variation under well-watered conditions (Figure S3A). Under drought stress conditions, PC1 explained 34.1 % of the variation, and PC2 explained 20.3 % of the variation (Figure S3B). At the anthesis stage, PC1 explained 34.2 % of the variation, and PC2 explained 18.3 % of the variation under well-watered conditions (Figure S3C). Under drought stress conditions, PC1 explained 40.4 % of the variation, and PC2 explained 18.9 % of the variation (Figure S3D). Under drought stress, modern cultivars were associated with proline at the stem elongation stage, but no distinct associations were detected under well-watered conditions. At the anthesis stage, the modern cultivars were associated with GY and Lic2 under both well-watered and drought stress conditions. During this stage, the genotypes of wild wheat and modern cultivars were clustered together within each of their status groups under both well-watered and drought stress conditions, whereas the distribution of landraces was scattered. However, at the stem elongation stage, the genotypes were generally more scattered; modern cultivars were an



**Fig. 3.** Grain yield per plant (A), aboveground biomass per plant (B), grain number per plant (C), thousand kernel weight (D), harvest index (E), and plant height (F). The unshaded circles indicate the mean relative values (drought/control) on the secondary vertical axis, and the genotypes are arranged in ascending order from low relative values (left) to high relative values (right). The white and black vertical bars indicate the mean values under well-watered and drought conditions, respectively, on the primary vertical axis. The data are average values of six replicates  $\pm$  standard errors. Asterisks above the vertical bar indicate significant differences between the well-watered and drought conditions (treatment effect) within the same genotype according to pairwise comparisons (\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ).

exception, as they formed a distinct cluster under drought conditions.

#### 4. Discussion

The high level of genetic diversity found in wild wheat relatives could prove useful in breeding programs (Kilian et al., 2021; Nevo and Chen, 2010; Sharma et al., 2021). In our study, we observed highly significant genotypic variations for all morphophysiological, biochemical, and yield-related traits in the *Triticum* genus that were measured during both the stem elongation and anthesis stages (Tables 2 and 3). Consistent with our results, several studies conducted on the *Triticum* genus have reported substantial genetic diversity for various physiological, morphological, and yield-related traits (Eser et al., 2024; Peleg et al., 2005; Reynolds et al., 2007; Saeidi et al., 2015; Suneja et al., 2019; Wang et al., 2017; Zaharieva et al., 2001). Mguis et al. (2008) reported that the genetic diversity observed for various traits is associated with geographical origin. This finding accords with our observations of

variations in the *Triticum* genotypes used in this study, which were collected from diverse regions. The high genetic variation across the measured traits in the *Triticum* genus offers promising resources for understanding and enhancing drought tolerance in wheat breeding programs.

##### 4.1. Drought stress affects physiological and biochemical traits during the stem elongation and anthesis stages

Our finding that gsw and RWC were significantly reduced due to drought stress during both the stem elongation and anthesis stages (Table 2) is consistent with the findings of previous studies, which reported reductions in gsw and RWC due to drought stress in wheat (Chauachi et al., 2023; Ru et al., 2023) and maize (Farman et al., 2022). Stomatal regulation is an important first line of response to drought stress in plants. Although a decrease in gsw is considered a drought avoidance strategy for maintaining the RWC, it also limits

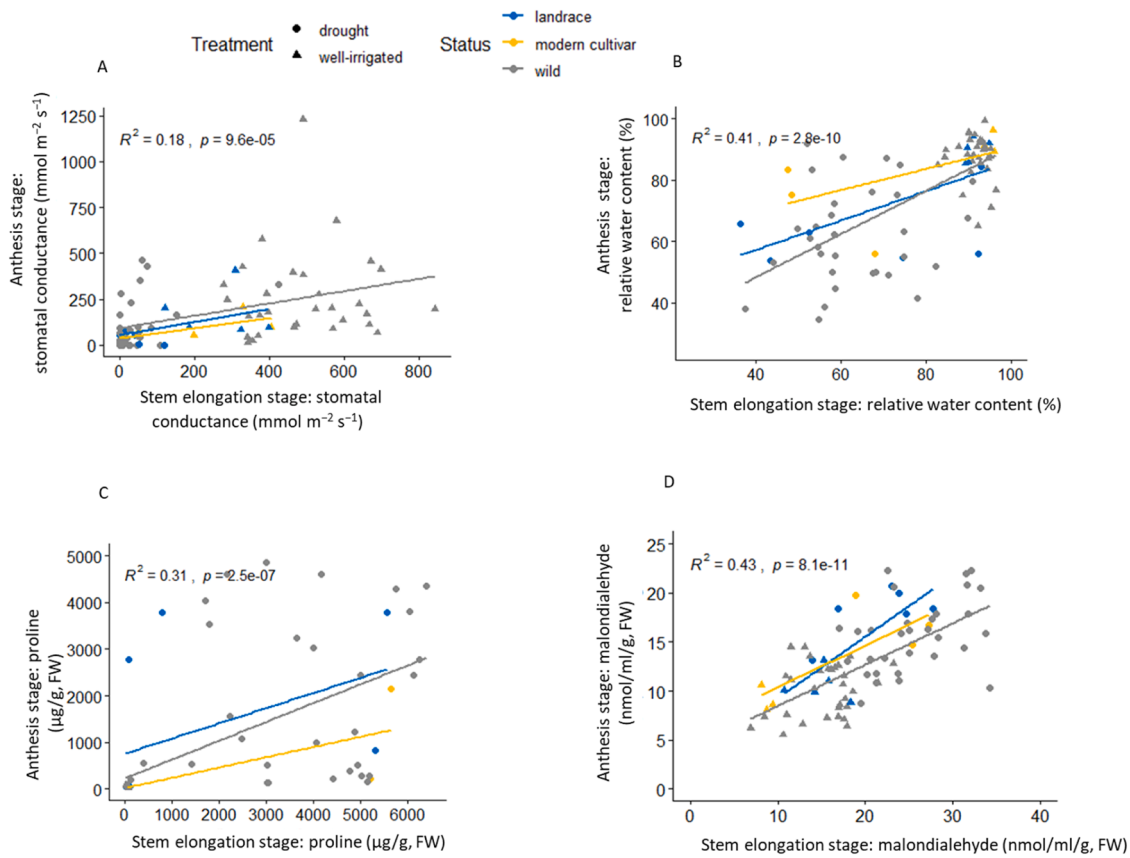


Fig. 4. Relationships between physiological traits at the stem elongation and anthesis stages. Stomatal conductance (A), relative water content (B), proline content (C), and malondialdehyde content (D). The blue, yellow, and gray lines and dots denote the landrace, modern cultivar, and wild status, respectively.

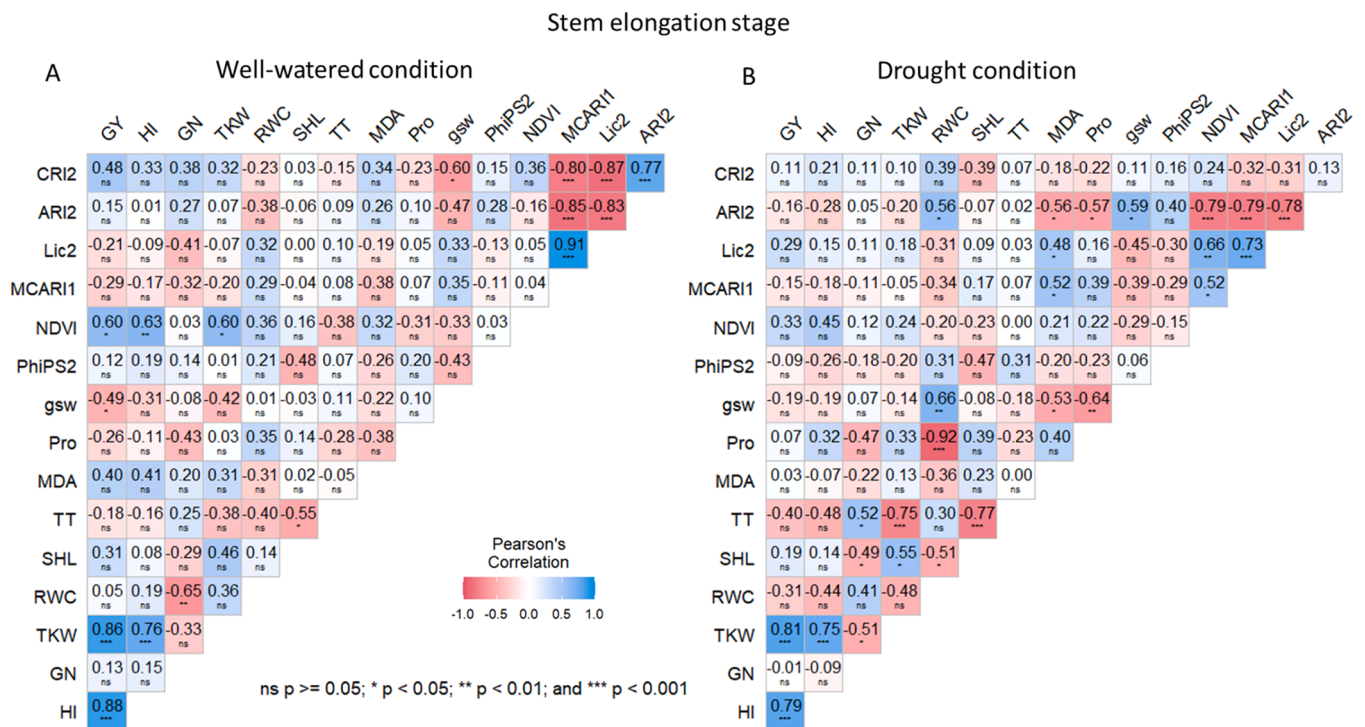
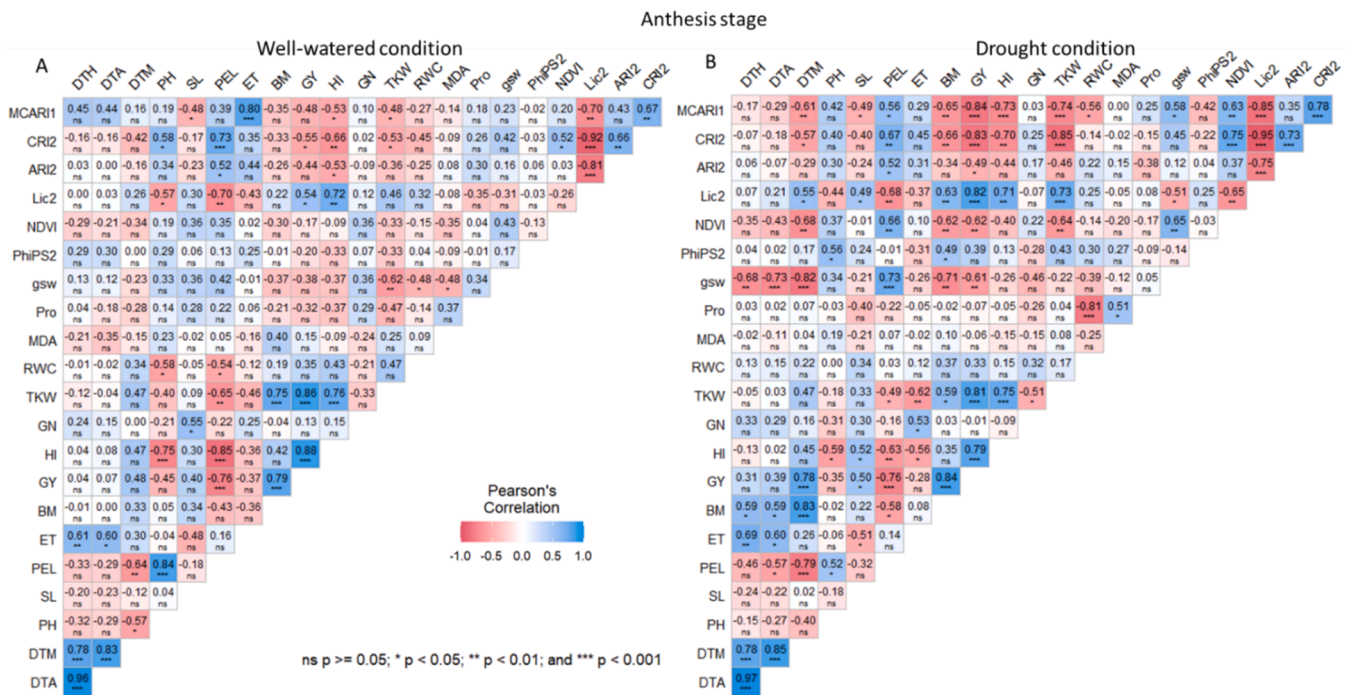


Fig. 5. Pearson's correlations between traits measured during the stem elongation stage under well-watered conditions (A) and drought conditions (B). AR12 = anthocyanin reflectance index 2, CR12 = carotenoid reflectance index 2, GN = grain number, GY = grain yield, gsw = stomatal conductance, HI = harvest index, Lic2 = Lichtenthaler index 2, MCAR11 = modified chlorophyll absorption reflectance index 1, MDA = malondialdehyde, NDVI = normalized difference vegetation index, PhiPS2 = photosystem II efficiency, Pro = proline, RWC = relative water content, SHL = shoot length, TKW = thousand kernel weight, TT = number of tillers.



**Fig. 6.** Pearson's correlations between traits measured during the anthesis stage under well-watered conditions (A) and drought conditions (B). ARI2 = anthocyanin reflectance index 2, BM = aboveground biomass, CR2 = carotenoid reflectance index 2, DTA = days to anthesis, DTM = days to maturity, ET = effective tillers, GN = grain number, GY = grain yield, gsw = stomatal conductance, HI = harvest index, Lic2 = Lichtenthaler index 2, MCARI1 = modified chlorophyll absorption reflectance index 1, MDA = malondialdehyde, NDVI = normalized difference vegetation index, PEL = peduncle exertion length, PhiPS2 = photosystem II efficiency, PH = plant height, Pro = proline, RWC = relative water content, SL = spike length and TKW = thousand kernel weight.

photosynthesis (Li et al., 2017). Moreover, the accumulation of MDA and proline contents (Table 2) can be interpreted as biochemical markers of drought stress (Kamruzzaman et al., 2023; Shrestha et al., 2022). During both the stem elongation and anthesis stages, the majority of the genotypes in this study demonstrated tolerance to oxidative damage. MDA is a lipid peroxidation product that indicates the level of oxidative damage in plants (Quagliata et al., 2023; Sallam et al., 2019). During the stem elongation stage, most of the genotypes showed significant accumulation of proline rather than MDA, suggesting that proline may have acted as an antioxidant. However, *T. durum* cv. Sambadur (G41) and *T. araraticum* (G221) revealed significant increases in both the MDA and proline contents, indicating increased stress levels in these genotypes. Therefore, our results revealed a genotype-dependent response to oxidative stress and proline accumulation. Proline accumulation in plants plays an important role in scavenging against oxidative stress, while also facilitating osmotic adjustment and stabilizing proteins and cell structures (Bandurska, 2022; Kavi Kishor and Sreenivasulu, 2014).

Plants experience osmotic stress when exposed to drought conditions. Therefore, maintaining a stable water content is essential for their optimal growth and development. We observed significant and negative correlations between proline content and RWC during both the stem elongation and anthesis stages under drought stress (Figs. 5B and 6B), which is in accordance with findings reported in previous studies (Marček et al., 2019; Saghour el idrissi et al., 2023). Furthermore, increased proline accumulation occurs in tissues with low water contents as opposed to those with high water contents (Chiang and Dandekar, 1995). Consequently, the high accumulation of proline in some drought-sensitive genotypes may be a symptom of stress rather than simply reflecting osmotic adjustments (Sanchez et al., 2012). Other researchers have argued that high levels of proline accumulation are linked to drought tolerance (Quagliata et al., 2023; Shrestha et al., 2022). However, our results revealed a significant treatment × genotype interaction for proline content and RWC during both the stem elongation

and anthesis stages (Table 2), indicating variations in genotypic responses to the treatment effects for these traits. These findings indicate that some genotypes effectively regulate proline accumulation and maintain RWC under drought stress conditions.

#### 4.2. Assessing genotypic variations in response to drought stress during the stem elongation and anthesis stages

Stomatal closure helps plants survive severe drought stress over longer periods (Gallé et al., 2013; Onyemaobi et al., 2021; Sade et al., 2012). However, not all the genotypes showed a significant decrease in gsw during the anthesis stage. Some shifts were observed during the anthesis stage, particularly in the *T. araraticum* (G161 and G189) genotype, which presented greater relative changes than during the stem elongation stage (Figs. 1A and 1B). Interestingly, these genotypes transitioned from being water-saving (isohydric) to becoming water-wasting (anisohydric) between the two stages, showing dehydration tolerance despite a significant decrease in RWC. This mechanism enables the maintenance of carbon gain during anthesis and grain filling, but it could increase the risk of hydraulic failure under severe drought stress (Scholz et al., 2012). The significant negative correlation between gsw and MDA under drought stress conditions during the stem elongation stage indicates that stomatal closure limits CO<sub>2</sub> assimilation, which triggers excessive oxidative stress in plants. However, no such association was observed during the anthesis stage, which may be attributed to the signaling effect that develops during the stem elongation stage. However, the reason for this phenomenon remains unclear.

Not all the genotypes responded significantly to drought stress in terms of RWC at both stages (Figs. 1C and 1D). Previous studies have shown that drought-tolerant cultivars exhibit a lower decrease in RWC than sensitive cultivars do (Cheng et al., 2016). In light of our results, the *T. monococcum* subsp. *sinskajae* (G89), *T. urartu* (G45), *T. araraticum* (G205), and *T. boeoticum* (G33) genotypes could be considered drought tolerant in terms of RWC at the stem elongation stage. *T. boeoticum*

(G27) revealed the highest level of drought tolerance, and only a few genotypes presented a significant decrease in RWC at the anthesis stage. Furthermore, *T. aestivum* (G10) showed the greatest susceptibility to RWC at the stem elongation stage, which significantly affected SHL and TT. Consequently, yield-related traits, namely, GY, BM, GN, and TKW, were also significantly affected in this genotype, which was therefore considered drought-susceptible. During the stem elongation stage, *T. araraticum* (G222) exhibited the lowest degree of response to drought stress for MDA, whereas *T. aestivum* cv. Nordkap (G181) showed the lowest degree of response during the anthesis stage, indicating avoidance of oxidative stress. During both stages, drought stress significantly increased the MDA content in *T. durum* cv. Sambadur (G41), indicating its susceptibility to oxidative stress. Previous studies have reported that wild wheat species such as *T. dicoccoides*, *T. boeoticum* and *T. urartu* are candidates for drought tolerance (Pour-Aboughadareh et al., 2017; Suneja et al., 2017). However, those studies did not address the impact of consecutive drought stress during both the vegetative and reproductive stages in wild wheat relatives. Furthermore, abiotic stress tolerance traits, including stomatal conductance, have rarely been investigated in *T. araraticum*. To our knowledge, only Sultan et al. (2012) included a single genotype of *T. araraticum* for studying physiological traits such as the MDA content, RWC and proline content and reported that it is susceptible to drought stress. In contrast, in our study, some genotypes of *T. araraticum* showed dehydration tolerance, indicating large variation in adaptability within *T. araraticum*.

Our results revealed a notable decrease in the relative value of MDA in *T. araraticum* (G221) and *T. boeoticum* (27) during the anthesis stage compared to the stem elongation stage. Preexposure of plants to environmental stresses can increase their ability to activate their defense mechanisms rapidly upon subsequent experiences of stress (Martinez-Medina et al., 2016). The decrease in MDA during the second drought episode suggested improved redox defense in the plants, which was likely due to the downregulation of cell membrane lipid peroxidation, which mitigated oxidative stress under drought stress (Abid et al., 2016). However, direct comparison of separate drought priming episodes during the two stages was limited by our experimental design: The same plants were subjected to drought treatments during both drought episodes, thereby confining our comparison of drought stress responses to the well-watered conditions at both stages. Our results revealed that, with a few individual exceptions, drought stress induced a lower increase in proline accumulation during the anthesis stage than during the stem elongation stage. Similar results have been reported for sugar beet, with a decrease in proline accumulation observed during subsequent drought episodes compared with its accumulation during initial stress events (Leufen et al., 2016).

During the stem elongation stage, ARI2 was negatively associated with MDA under drought stress (Fig. 5B, Figure S3B). Anthocyanins protect leaves from excess light and serve as scavengers of reactive oxygen species (Sims and Gamon, 2002). The increased efficiency of plant photosystems under drought stress helps prevent oxidative damage (Abid et al., 2016). This effect was also demonstrated by the positive association between ARI2 and RWC in our results (Fig. 5B, Figure S3B), indicating that photoprotection may influence plant hydration. Moreover, we found a weaker significant positive association between the stem elongation and anthesis stages for biochemical and physiological traits, excluding vegetative indices (Fig. 4, Table S3). The response of plants to drought stress is influenced by their growth stage, age, species, or genotype and by the duration of drought stress (Gray and Brady, 2016). Therefore, the physiological and biochemical mechanisms involved in drought tolerance remain complex. Moreover, the trait associations in this study may have been masked by the high genotypic variation. Our results suggest genotype-dependent responses to drought stress across the developmental stages of plants.

#### 4.3. Comparative analysis of drought adaptation mechanisms in wild wheat relatives and modern wheat cultivars

In our study, proline accumulation at the anthesis stage did not significantly differ according to treatment effect across status groups (Table S2). Our results revealed significant treatment effects for RWC and MDA in the wild-type group but no clear trait dominance favoring the wild-type status for drought tolerance. Instead, we observed a genotype-specific response for these traits (Figs. 1D and 2B). However, a previous study (Wang et al., 2017) reported stronger biochemical responses to drought tolerance in modern hexaploid cultivars than in landraces and wild wheats, but that study included different sets of genotypes from domesticated *Triticum* and wheat wild relatives belonging to *Aegilops tauschii*.

Our findings of greater reductions in PH and gsw under drought stress in wild plants and landraces (Table S2) indicate that drought avoidance strategies are more effective in wild wheat relatives and landraces than in modern cultivars. The significant negative correlation between gsw and GY under drought stress in this study indicated that drought avoidance contributes positively to GY. This finding contradicts previous findings of a positive association between gsw and GY under stress (Fischer et al., 1998; Zheng et al., 2011). A meta-analysis (Li et al., 2021) revealed that drought tolerance via osmotic adjustment and the antioxidant defense system did not lead to increased yield under severe drought stress in wheat, which is consistent with our finding of no correlation between RWC or proline content and GY or other yield-related traits. These findings suggest that complex physiological and biochemical mechanisms contribute to the GY under severe drought stress. These may have low heritability (Chen et al., 2012) and therefore low breeding value. Although no correlation was detected between proline content and yield (Fig. 5 and Fig. 6), the drought-tolerant genotypes reported in our study possibly benefited from drought-induced proline accumulation, which occurred in all the studied genotypes under drought stress (Figs. 2C and 2D).

We observed accelerated heading, anthesis, and maturity (Table 3). Phenological adaptation is a drought escape mechanism and an important factor influencing GY in plants (Foulkes et al., 2007; Shavruk et al., 2017). Plants with longer phenological durations are prone to drought stress. A short anthesis and grain-filling period induced by drought stress reduces GY in wheat (Eser et al., 2024; Lv et al., 2017). However, we observed a yield advantage due to early anthesis induced by drought stress in certain genotypes. For example, the *T. dicoccoides* (G242) genotype exhibited a drought escape mechanism via early anthesis under drought stress but did not significantly reduce GY or GN; instead, it significantly increased HI. Additionally, *T. monococcum* subsp. *sinskajae* (G89) and *T. boeoticum* (G27) revealed a drought escape mechanism. These findings suggest that these genotypes may have employed drought escape mechanisms to mitigate the negative effects of drought stress, while they activated the physiological grain formation process after watering recommenced to maintain the GY under drought stress. In line with previous findings (Lopes et al., 2012), we found a positive correlation between DTM and GY under drought stress, but no correlation was detected between DTH or DTA and GY, averaged across all the genotypes. These findings reveal a complex interplay between phenological responses and yield components under water stress in the *Triticum* genus. To some extent, our observations of responses to drought stress across the status groups are aligned with those of a previous study (Li et al., 2021), which showed that some wild wheat relatives and landraces in the *Triticum* genus exhibit drought avoidance and escape mechanisms. Consequently, breeding for drought adaptation in wheat should also incorporate avoidance and escape mechanisms together with drought tolerance (Mohammadi, 2018; Sallam et al., 2019). In our study, *T. boeoticum* (G27) demonstrated all three mechanisms for drought adaptation: drought escape, avoidance, and tolerance via enhanced anthesis; a high response to gsw and PH; and a low reduction in RWC at the anthesis stage. Similarly, *T. monococcum* subsp. *sinskajae*

(G89) exhibited a drought escape mechanism via early anthesis, and among the genotypes with a lower reduction in RWC, it demonstrated drought tolerance at the stem elongation stage. *Triticum dicoccoides* (G242) evokes a drought escape mechanism via enhanced anthesis and drought tolerance via high RWC at the anthesis stage. In our previous field experiment, *T. dicoccoides* (G242) and *T. boeoticum* (G27) also showed drought adaptation, with high responses to gsw, and *T. monococcum* subsp. *sinskajae* (G89) exhibited a low response to gsw while maintaining yield under rainfed conditions (Pantha et al., under review). The underlying drought adaptation in these genotypes can be attributed to the distinct semiarid to arid climates of their native regions in the Fertile Crescent, characterized by hot, dry summers and water scarcity, which pose key environmental constraints (Jaradat, 2017). These genotypes can be utilized as sources of drought adaptation traits. In our experiment, not all the wild genotypes and landraces showed drought tolerance; however, the drought-tolerant candidates identified were primarily from wild wheat and landrace groups, which were sourced from our own collection and seed multiplication efforts. Modern wheat varieties exhibit narrow genetic diversity for drought tolerance; therefore, wild wheat relatives and landraces may be promising natural sources for additional drought tolerance genes and mechanisms (Sharma et al., 2021). Therefore, this study is highly relevant for biological research and food security. Moreover, this study highlights the importance of integrating different drought adaptation mechanisms to achieve stable yields under severe and frequent drought stress, particularly at the stem elongation and anthesis stages. Previous studies have emphasized that drought tolerance aligns with yield (Blum, 2017; Wang et al., 2017). However, they did not account for the contribution of drought avoidance mechanisms to yield under severe drought stress. Our findings showed that drought avoidance can possibly contribute to yield in specific genotypes under severe and frequent drought stress conditions. Therefore, the potential ability of a specific genotype to adapt to varying degrees of drought stress should be emphasized (Turner et al., 2007).

We used *T. dicoccoides* genotypes from two different genetic taxa: *T. dicoccoides* (G242), a western race from Israel, and *T. dicoccoides* (G90 and G248), a central-eastern race from Turkey and Iran (Özkan et al., 2011). In our study, the western race presented greater tolerance at anthesis, whereas the central-eastern race presented greater tolerance at the stem elongation stage, with relatively greater RWC.

#### 4.4. Morphological and yield component plasticity in the investigated genotypes

Our results revealed that drought stress negatively impacted morphological and yield-related traits, notably PH, SL, PEL, ET, BM, GY, and GN (Table 3). Among these traits, GY, averaged across all the genotypes, presented the greatest reduction. Our results revealed a significant reduction in the GY of 51 %, which is consistent with a previously reported decrease in yield of 53 % in wheat under severe drought stress (Ahmed et al., 2020). In light of the relative performance of GY in our study, the *T. monococcum* subsp. *sinskajae* (G89), *T. boeoticum* (G27), and *T. dicoccoides* (G242) genotypes showed the greatest drought tolerance. Furthermore, the maintenance of GY under drought stress in these genotypes was attributed to various yield components and morphological traits, each contributing differently to different genotypes. The drought tolerance of cereal crops is characterized primarily by their ability to maintain stable yields under drought stress conditions and how they respond to each yield component (Bandurska, 2022; Wang et al., 2017). Decreased competition for resources allocated, for example, to PH and BM, could increase the allocation of resources to spikes, which would contribute to increasing the HI (Foulkes et al., 2011). Our results revealed a significant negative correlation between PH and HI, which suggests a trade-off between the allocation of resources for PH and grain sink strength. On the one hand, a drought-tolerant genotype, *T. dicoccoides* (G242), showed significantly decreased PH and BM and consequently a significantly increased HI,

although the GN decreased significantly. The drought-tolerant genotypes *T. monococcum* subsp. *sinskajae* (G89) and *T. boeoticum* (G27) stabilized GY through the maintenance of GN. Grain number and seed weight are related to grain sink strength (Miralles and Slafer, 2007; Sadras and Slafer, 2012; Slafer et al., 2023). Our results indicated that GN was negatively correlated with TKW, suggesting a trade-off between resource allocation for grain size and grain number, as reported by Quintero et al. (2018).

The limited tillering ability of plants under water stress conditions is advantageous for efficiently conserving water and could contribute to increased yield-related traits (Richards et al., 2010). This is supported by our finding of a significant negative correlation between TT and TKW during the stem elongation stage under drought stress conditions. Furthermore, we observed a significant negative correlation between ET and HI with TKW only under drought stress conditions. However, SL was positively correlated with GY under drought stress conditions but not under well-watered conditions. Chen et al. (2012) reported that SL is one of the major traits for drought tolerance and contributes to GY in wheat under water stress conditions. Therefore, our results confirm that reduced PH and tillers may be advantageous for efficient water conservation during the grain-filling stage and highlight the significant contribution of SL to maintaining GY under severe drought stress conditions.

In general, climate-controlled greenhouse pot experiments are used to study complex drought tolerance mechanisms accurately, minimizing external factors to ensure consistent results (Poorter et al., 2016). However, they limit root growth, which is a key factor for drought tolerance, and other external environmental factors that occur under field conditions. Therefore, we propose further confirmation in field conditions with their inherent variability, including fluctuating environmental factors and complex interactions.

## 5. Conclusion

Genotype-dependent responses to drought were observed during the stem elongation and anthesis stages, with some genotypes exhibiting drought tolerance during both stages. Our first hypothesis that drought tolerance mechanisms for osmotic and stomatal regulation are correlated between the stem elongation and anthesis stages was partially confirmed by the results. Certain genotypes that adapt to drought through different mechanisms were able to maintain yield stability under drought stress, although traits such as proline content, RWC and MDA content were not associated with GY, which might be due to the highly variable genotypes used in this study. The results related to our second hypothesis, namely, that wild wheat relatives exhibit superior drought tolerance compared with modern wheat cultivars, were inconclusive. We observed genotype-dependent responses to drought stress, revealing trait diversity in the studied *Triticum* gene pool and identified candidates for drought tolerance, which can be utilized in wheat breeding programs. These candidates exhibit specific physiological and biochemical traits that could increase drought tolerance, contributing to yield. Therefore, exploiting different mechanisms of drought adaptation, such as drought avoidance, drought escape, and drought tolerance, in breeding programs is crucial for enhancing yield stability under drought conditions. Our study contributes to a substantial expansion of the gene pool that can provide traits in wheat improvement programs aimed at drought adaptation.

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### CRedit authorship contribution statement

**Sumitra Pantha:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Benjamin Kilian:** Writing – review & editing, Conceptualization. **Hakan Özkan:** Writing – review & editing, Conceptualization. **Frederike Zeibig:** Writing – review & editing, Investigation, Conceptualization. **Michael Frei:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

### Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. Sumitra Pantha reports financial support was provided by German Academic Exchange Service. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.envexpbot.2024.106047](https://doi.org/10.1016/j.envexpbot.2024.106047).

### Data availability

All data relevant to this article can be found in the article and in the supplementary material.

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